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TECHNICAL COMMUNICATION No. 16

FURTHER WORK ON PLANT INJECTION FOR DIAGNOSTIC AND CURATIVE PURPOSES

by
W. A. ROACH and W. O. ROBERTS,
East Malling Research Station

August, 1945

Price 1/6

Reprinted by courtesy of the editors of the "Journal of Pomology and Horticultural Science," from J. Pomol., April 1945, Vol. XXI, pp. 108-119, and published by the Imperial Bureau of Horticulture and Plantation Crops, East Malling, Kent, England. Obtainable from I.A.B., Central Sales Branch, Penglais, Aberystwyth.

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FOREWORD

In 1938 Dr. Roach surveyed the whole field of plant injection work and gave details of the technique used by himself and others in Technical Communication No. 10.

Since then not only has there been a persistent and increasing demand for the T.C. and for relevant information, but also considerable advance in technique has been made by the workers in this field.

By now, therefore, under normal circumstances the bureau would have hoped to bring the whole subject up to date by a further comprehensive survey, but this the stress of war has prevented.

Hence, it has seemed reasonable to do the next best thing, namely, to offer the present paper by Dr. Roach and Mrs. Roberts, in which they describe the improvements in technique introduced by the East Malling team since 1938, as a useful addendum to the information given previously. The paper considered as an interim survey, should, it is thought, fill the gap until such time as a fuller survey is possible.

The Bureau is most grateful to the authors and to the Editors of the *Journal of Pomology* for permission to reprint the paper and make it readily available at a low price to those interested.

FURTHER WORK ON PLANT INJECTION FOR DIAGNOSTIC AND CURATIVE PURPOSES

By W. A. ROACH and W. O. ROBERTS

East Malling Research Station, Kent

THIS communication describes improvements made in plant injection methods since they were described in detail (Roach, 1938, 1939) and includes experience on a wider range of plants, both in this country and in South Africa. A brief note on the results obtained has already been published (Roach, 1940). The methods elaborated are now in use in many parts of the world, but the present is not a suitable time for making a comprehensive survey of the recent work of other investigators; this is deferred until after the war. The present account is written as an addendum to the earlier papers, to which page references are given.

I. INJECTION FOR DIAGNOSTIC PURPOSES.

Concentration of solutions.—Responses have been obtained on a number of different kinds of plants with the following solutions, but it may be necessary to modify them, e.g. when work is done on new kinds of plants.

N	1%	Urea.
P	0·5%	Sodium dihydrogen phosphate.
K	1%	Potassium chloride.
Ca	1%	Calcium chloride.
Mg	0·5%	Magnesium sulphate.
Fe	0·025%	Ferrous sulphate+0·025% (by volume) sulphuric acid.
Mn	0·025%	Manganese sulphate+0·025% (by volume) sulphuric acid.
Zn	0·025%	Zinc sulphate+0·025% (by volume) sulphuric acid.
Cu	0·025%	Copper sulphate+0·025% (by volume) sulphuric acid.
Ni	0·025%	Nickel sulphate+0·025% (by volume) sulphuric acid.
B	0·1%	Boric acid.

High grade chemical reagents are quite satisfactory. The sulphuric acid is added to the metallic trace element solutions to prevent all risk of precipitation by hydrolysis. This has been a serious inconvenience in respect to the ferrous sulphate solution.

A. INTERVEINAL LEAF INJECTION (cf. Roach, 1938, p. 18; 1939, p. 168).

Further experience with this method has confirmed the conclusion that a response may be detected more rapidly by it than by any other method. In an experiment by B. N. Lal (1944) at Malling a response to iron was observed in the Soya bean two days after injection. The method as originally described has, however, two serious drawbacks: it calls for delicacy of manipulation and it cannot be used in windy weather.

The apparatus described below (Fig. 1) has made manipulation easier, has removed the tendency for the contrivance to fall before the absorption is complete, and has increased its resistance to wind. The improvements consist mainly in the replacement of the long, relatively heavy, glass tube by a short, light one, made of cellophane, and the use (in one method) of a chisel-ended scalpel to make an incision of exactly the right width, the lips of which consequently clip the filter paper wick and hold it firmly.

Construction of the appliance.—The cups for holding the liquids to be injected are made from cellophane drinking straws (those without a decorative strip of tinsel). These are obtainable in several distinctive colours, which may if desirable be increased by marks in waterproof

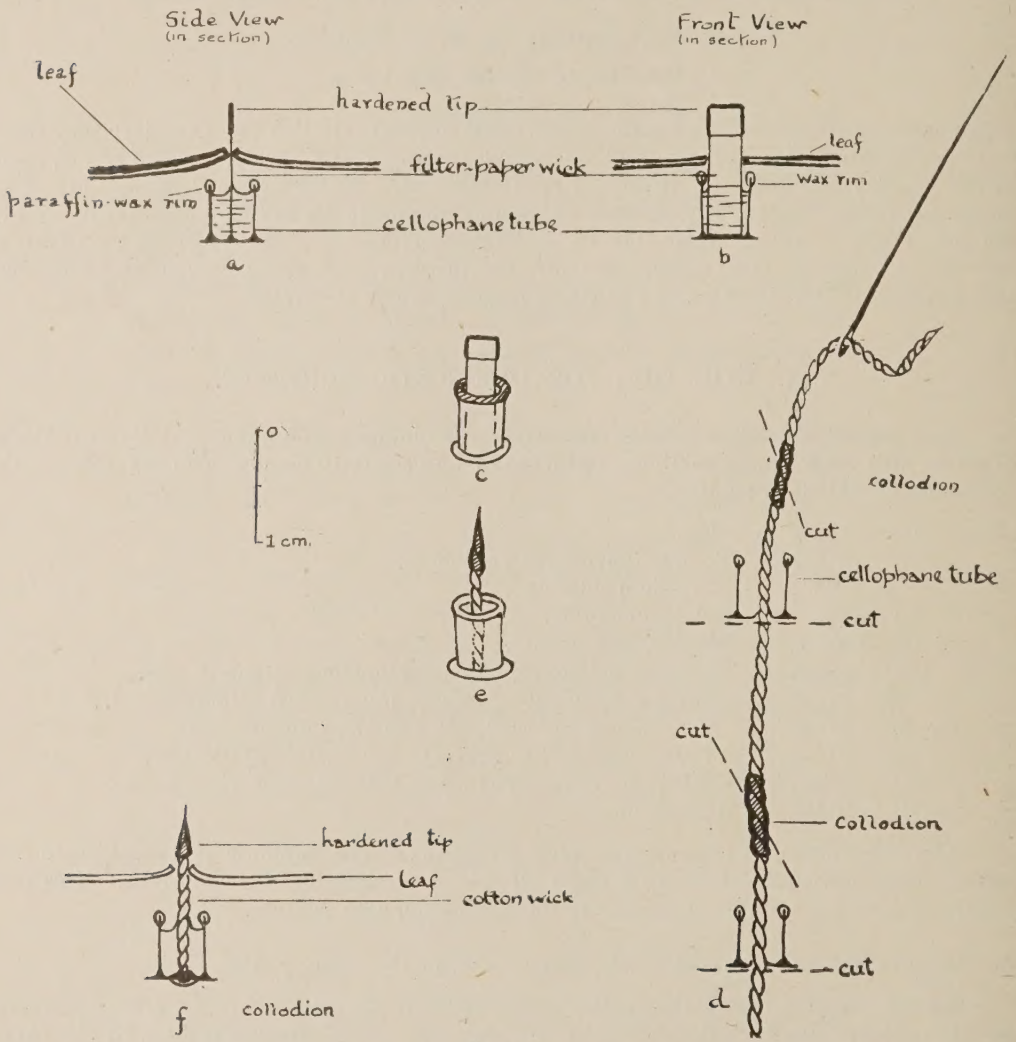

FIG. 1.

Apparatus for interveinal injection. Side and front sectional views of one form in use are seen in (*a*) and (*b*). It is drawn in the solid in (*c*). The manufacture of the other form is illustrated in (*d*). It is seen in the solid in (*e*), and (*f*) is a sectional view of it in use.

ink. If each colour or colour combination is used for a particular solution the little cups may be used a number of times without cleaning.

The straws are cut to the required length with the apparatus illustrated in Fig. 2. The straws are slid along the guide A, through the hole B in the vertical piece of box-wood C up against the stop D, which can be adjusted to give different lengths of straw, the length being

read on the protruding end E of the stop. A new safety-razor blade is mounted inside the wooden slide F, the component parts of which are shown in *b*, *c* and *d*. These components are fitted together so that the bottom edge of the middle part, shown in *c*, occupies the position shown by the dotted line in *d*. The safety-razor blade is held in a sloping position by two dowel pins G and H protruding from the side-piece shown in *d*, passing through the holes in the blade and through similarly lettered holes in the middle *c* and the other side-piece *b*. The three pieces of wood are held together by three nuts and bolts, J, K, L. When in use, the blade is thus held tightly against the side of C. When the slide is moved towards the straw along C the blade passes across hole B, cleanly cutting off the protruding length of straw without denting it, and it is caught in a suitably placed shallow box.

To lessen the tendency of the liquid to be drawn out by capillarity along the leaf surface when the underside is hairy, the rim of the cup is protected by a thin layer of paraffin wax (Fig. 1, a & b). The layer is applied by heating a little of the wax on a horizontal sheet of glass over a steam bath and dipping one end of each piece of tube into the thin layer of melted wax.

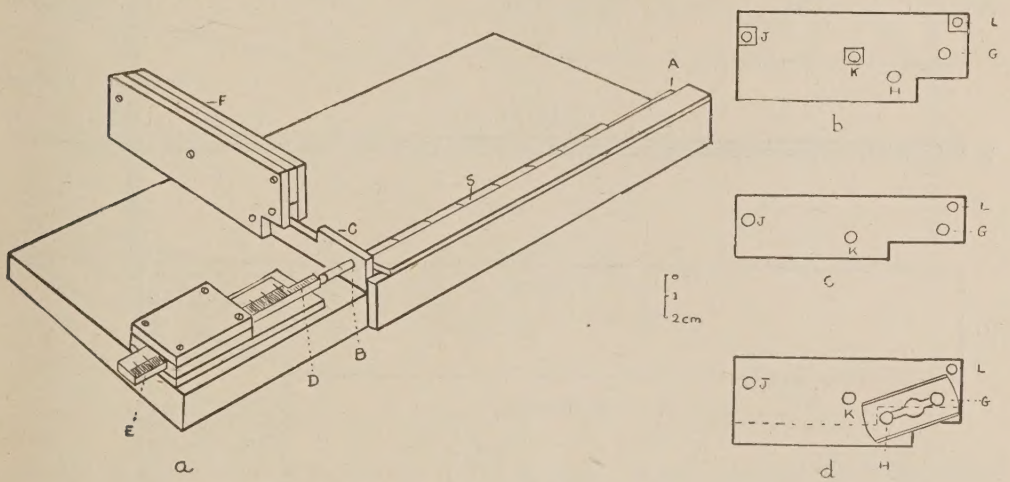

FIG. 2.

(*a*) Guillotine for cutting cellophane drinking straws into lengths suitable for injection cups. (*b*), (*c*), (*d*), details of F. For detailed description see text.

Discs to form the bottoms of the cups are cut from sheet cellophane with a leather-cutting punch of slightly greater diameter than that of the tube, a piece of card being placed between the cellophane and the brass block against which the cutter is pressed. Into the non-waxed end of each bit of cellophane tube is squeezed a drop of collodion* and the tube is then placed over one of the discs so that the collodion may seal the two together, thus closing the end of the piece of tube and forming a little cup. While the collodion is still liquid a filter paper wick is placed in the cup. When the cup is dry the tip of the wick is hardened by brushing it with a little dilute collodion. This soaks in and hardens about 2 mm. of the wick. Instead of a filter paper wick a cotton one may be used. For that purpose the cups are allowed to dry, ordinary unmercerized mending cotton is drawn up through the bottom of the cup with a very fine darning needle (Fig. 1, *d*, *e*, *f*). The cotton is cut off just below the bottom of the cup and a little longer than is necessary above. Collodion is squeezed over the bottom of the cup to secure the thread and seal the hole ; it is also smeared over the protruding end of the thread, leaving about 2-3 mm. untreated above the rim of the tube. When quite dry, the hardened end is

* An adhesive sold in convenient tubes and known as Durofix, is suitable for this purpose.

sharpened to a point by cutting slantwise with a razor blade, as shown in Fig. 1 (*d*), which also shows how several of such tubes may be provided with cotton wicks at one and the same time.

Both types of cup can be filled with the required liquids by using an ordinary dropping bottle with a slender delivery tube.

The scalpels (Fig. 3, A and B) are made by breaking a suitable piece from a thin safety-razor blade of the Gillette type held in parallel-jawed pliers and cementing it between two flat wooden spills with collodion. A number may be clamped between boards to dry overnight, after which collodion is run around the joint between the blade and the wood to strengthen it.

For work in the field it has been found convenient to carry the various items of the apparatus in a box fitted so that each dropping bottle occupies its own compartment in a row and has opposite it two compartments, one for each type of its distinctively coloured injection cups.

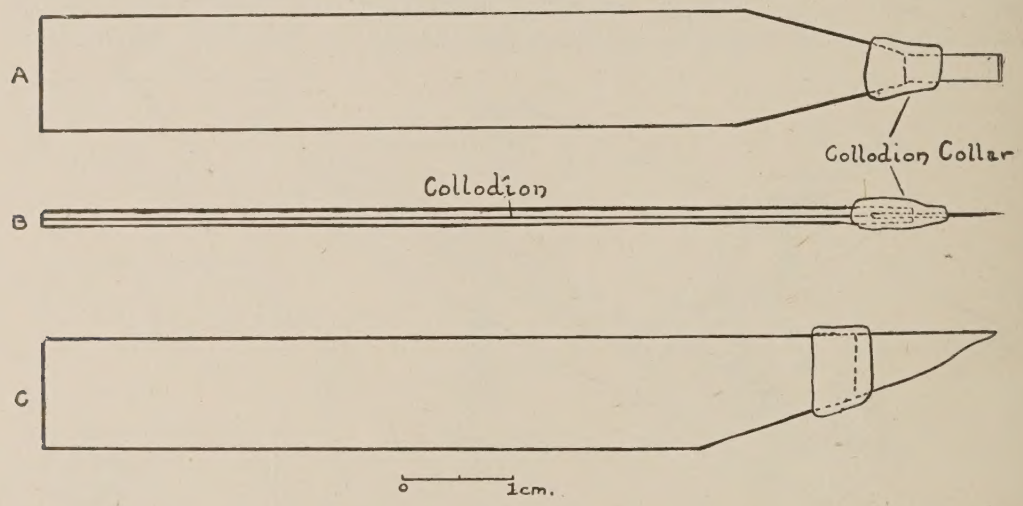

FIG. 3.

Scalpels suitable for diagnostic injection. A, front, and B, side view of chisel-end scalpel for interveinal injection. C, front view of scalpel for leaf-stalk injection.

B. LEAF-STALK INJECTION (cf. Roach, 1938, p. 27 ; 1939, p. 180).

This is by far the most generally useful of all diagnostic injection methods. It is at present in routine use for testing the effect of essential elements singly and in combination, and many thousands of injections of this type have been done. Experience has proved the desirability of carrying out the test with each solution at least in quadruplicate. Usually about a dozen elements are involved, which entail about fifty injections. Water injections should also be included.

Construction of apparatus.—The apparatus found most convenient is shown in Fig. 4, A and B, and two methods of using it are illustrated in Fig. 5, A and B. Apparatus shown in Fig. 4, B is for use with smaller leaves.

For lightness, tubes are blown from the thinnest walled quill glass tubing. These are obtainable from Messrs. C. L. Muller, 6 Parton Street, Red Lion Square, London, W.C.1.

A suitable length of lead wire is held against the tube in a spring clothes peg. Collodion is applied to the bottom of the lead wire so as to stick it to the tube ; a little more is applied to the wire near the open end of the tube. While the collodion is still liquid two turns are made round both ends of the tube with thread, which is then covered completely with a layer of

collodion. When dry the clothes peg is removed and collodion is applied along the whole length of lead wire in contact with the tube. In this way the exterior of the whole contrivance is left quite smooth and without projections which might scratch the leaves in a wind. Finally, a thin band of melted paraffin wax is painted round the inner rim of the tube. This tends to prevent the liquid from running out of the tube, even when it slopes mouth downwards.

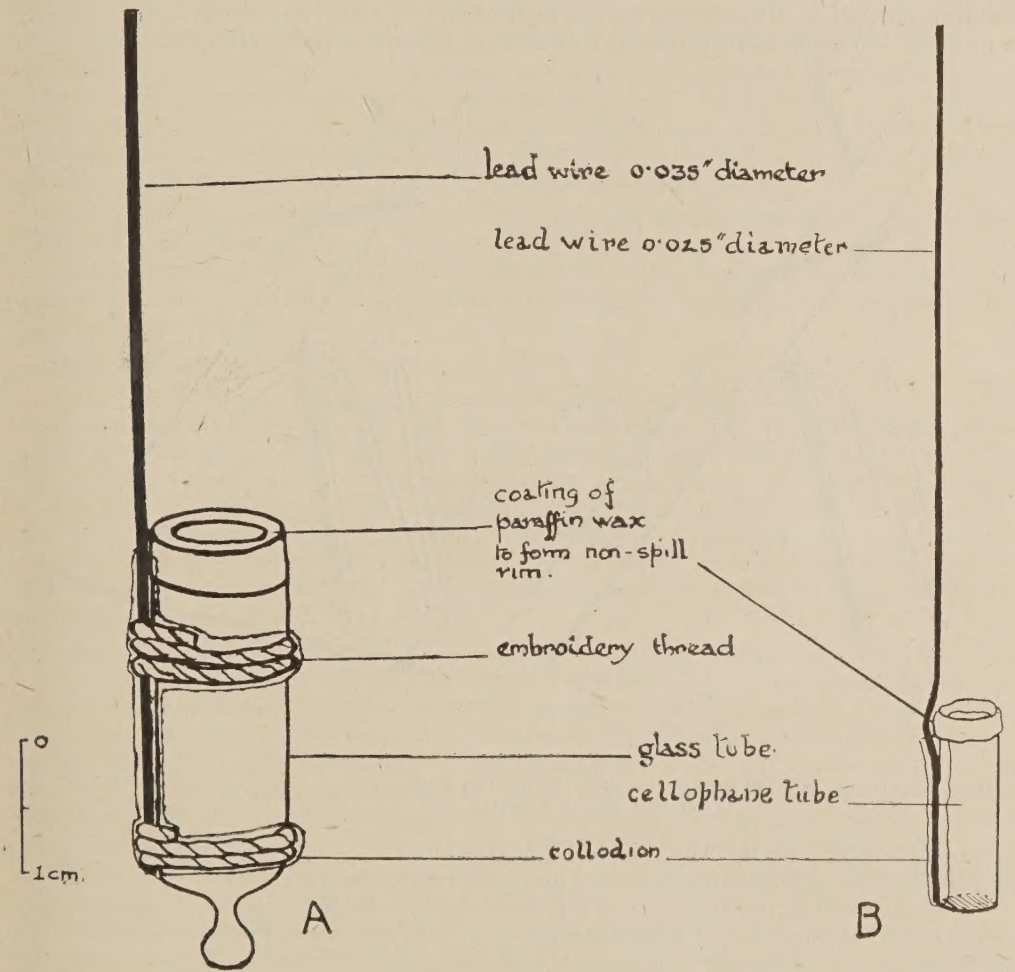

Fig. 4.
Cups for leaf-stalk injection. A for larger and B for smaller leaves.

For slender leaf-stalks the single coil of the wire shown in Fig. 5, A is sufficient to bend the stalk down. Stiff leaf-stalks can be bent down by using a piece of ·025 in. diameter lead wire attached to the basal knob of the tube and with its other end coiled around the stem of the plant (Fig. 5, B). Both for maximum speed in working and for minimum damage to the plant the wire should be twisted as little as possible.

The type of scalpel found most convenient for leaf-stalk injections is shown in Fig. 3, C.

It is easily held in the mouth, or even stuck to the moistened underlip, between operations, and its lightness is a distinct advantage in use.

As already pointed out (Roach, 1938, 1939), the reliability of a diagnosis made by the leaf-stalk injection method depends on how closely the distribution of the improvement (if any) in leaf colour (and sometimes the growth increase) corresponds with expectation based on experiments with dyes, and on the degree of likelihood of a similar distribution of colour variation occurring naturally. Hence before starting such injections on a fresh type of plant it is necessary to do a sufficient number of experiments with dyes and to make a survey of the naturally occurring

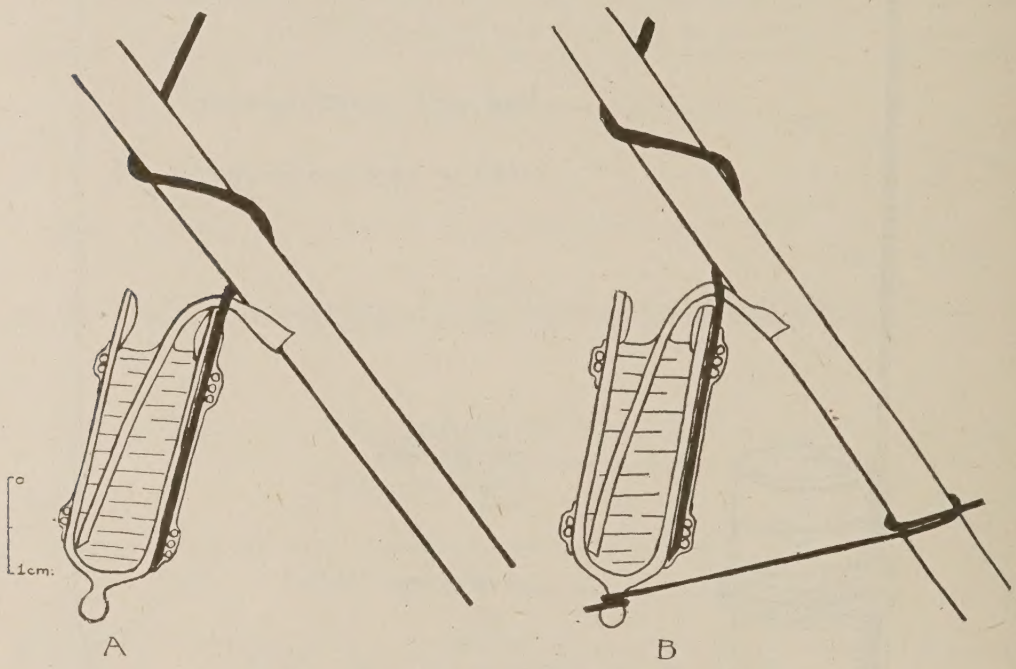

FIG. 5.

Method of attaching injection cups to the plant stem. The method shown in A is used unless the leaf-stalk is too stiff, when the method seen in B is used.

variations in colour and size which might be mistaken for the result of the experiment ; shoots with leaves showing such variations must, of course, be avoided.

In such plants as the apple, the distribution of injected liquids may be predicted within narrow limits, and there are all stages between this type of material and the aubergine, in which the distribution is so variable as to make this type of injection useless for diagnosis. There are similar differences between species of plants in respect of the degree of variation in colour and size of the leaves. The degree of variation in the leaves of a given plant depends largely on the degree of uniformity of the soil.

C. SUMMARY OF EXPERIENCE WITH VARIOUS PLANTS.

Extensive injection work has been done for the most part on relatively few kinds of plants but enough has also been done on a number of other kinds to suggest the most hopeful methods to try on them. The following notes summarize our present knowledge. The plants are arranged alphabetically.

Apple.—Frequent references have been made to methods suitable for the apple (Roach, 1938, 1939). The leaf-stalk method is the most generally useful. The commonest type of distribution of injected substance has already been described (Roach, 1938, pp. 27-29 ; 1939, pp. 181-183). The only really commonly occurring divergence from this pattern is one in which the two leaves immediately above and below the injection point respectively do not become permeated. In a recent experiment with 1 per cent. acid fuchsin on the variety Lord Derby, in which 100 shoots were injected, the first leaf below the injection point in 10 shoots was free of dye, and in one shoot only were the leaf below and the leaf above this point free of dye.

Apricot.—The distribution resulting from the injection of a regular shoot of the apricot with dye solution by the leaf-stalk method is similar to that shown in Fig. 19, Roach, 1938 and text Fig. 17, Roach, 1939. Judging from the small number of shoots injected, it would seem that shoots in which the arrangement of the leaves is irregular is somewhat common. These must be avoided, because they give unpredictable injection patterns.

Aubergine.—Dye solution injected into the aubergine by the interveinal method moves freely, and even medium sized veins do not form definite barriers to permeation, but the method seemed worth trying. The plants used were unusually healthy, however, and no response was obtained to mineral injection. The distribution resulting from leaf-stalk injection was irregular and this method appeared useless for diagnosis.

Broad Bean.—See Hill and Roach, 1940, pp. 518-520.

Cape Gooseberry.—A few experiments carried out late in the season with vigorous Cape Gooseberry plants below deciduous fruit trees in the Western Cape Province, South Africa, suggest that this plant would lend itself to diagnosis by the leaf-stalk method, and that the first, second and third leaves above the injection point become permeated sufficiently unequally on their two sides to be useful for diagnosis. Strong colour responses and obvious growth increases were obtained. Shoots in which the arrangement of the leaves is not quite regular must be avoided. Although not of great commercial importance this plant is nevertheless worth testing as an indicator plant, especially for diagnosis when the fruit trees in the same plantation are not in growth.

Citrus.—A few experiments were carried out on orange trees between the seasons of growth flush. These suggested that the leaf-stalk method is well worth trying on trees making vigorous growth. An abnormal type of shoot common on trees between growth flushes must be avoided, because leaf-stalk injection of them results in a general distribution of the injected liquid throughout the whole shoot. These shoots are easily recognized because their leaves are small and become progressively smaller from the base of the current year's growth to the tip. The experiments done on normal shoots suggest that the distribution may be predicted with sufficient accuracy for diagnosis. The commonest type of distribution corresponds closely to that given by the pear (Roach, 1938, p. 33 ; 1939, p. 188). Citrus leaves often become bleached in sunlight and this must be borne in mind in making a diagnosis. No positive response was obtained in any of the few score injections of nutrients carried out during a visit to the Union of South Africa, but none of these was done under the best conditions for getting a response. Experiments under better conditions, i.e. when the trees are making vigorous growth, are therefore highly desirable.

Coffee.—See Roach, 1938, p. 35 ; 1939, p. 189.

Currant.—See Roach, 1938, p. 33 ; 1939, p. 188.

Gooseberry.—See Roach, 1938, p. 33.

Gousblom (Arctotis).—Work was done on this plant (Compositae) because it is a self-sown cover crop commonly found in some of the orchards of the Western Cape Province and it is in vigorous growth when the fruit trees are leafless. The pattern resulting from leaf-stalk injection resembles that of the apple.

Guava.—Experiments with dyes only have been done on this plant. Interveinal injection results in the permeation of the area without any dye crossing the bounding secondary veins. Leaf-stalk injection gives the same distribution as in coffee and the Hydrangea.

Hop.—See Roach, 1938, p. 35.

Japanese Plum.—The leaf-stalk method is the most useful one for this plant and it gives the same distribution as in the common plum. Care must be taken, however, not to use shoots developing from adventitious buds on old wood, because leaf-stalk injection on such shoots results in permeation of the leaves of practically the whole shoot ; no leaves became permeated on one side of the midrib and not on the other.

FIG. 6.

The distribution of liquid resulting from the injection of a lupin leaf through a cut leaflet tip.

Loquat.—Experiments with dyes only have been done on this plant. The extreme hairiness of the loquat leaf does not interfere with interveinal injection because the hairs are not easily wetted. Dye solution injected through an interveinal incision permeates the interveinal area thoroughly, and, in addition, enters a band near the midrib nearly reaching the leaf tip. Comparison must therefore be made mainly between one side of the leaf and the other and not between contiguous interveinal areas. The effect of a high concentration may be seen by comparing the interveinal area containing the incision with the one on the opposite side of the leaf, and the effect of a lower concentration by comparing most of the injected half leaf with the other half. Leaf-stalk injection brings about the permeation of leaves of the whole shoot and can therefore be used for experiments in which a unit of this size is desired.

Lupin.—The leaflet-tip method of injection has been found to be applicable to a number of types of lupin grown as cover crops in South Africa and in gardens in this country. A typical result of injecting dye is shown in Fig. 6. The effect of injected nutrients is usually seen in the

shaded and black areas. Leaf-stalk injection tends to result in the second leaf above, the first and third leaves below, becoming permeated on the proximal but not on the distal side. Limited experience with this method suggested that it is worth trying as well as the leaflet-tip method. The leaf-stalk one is the easier to carry out.

Peach.—The leaf-stalk method of injection is the most useful and gives the same pattern as in the apple.

Pear.—See Roach, 1938, p. 33 ; 1939, p. 188.

Plum.—The leaf-stalk method of injection is the most useful and gives the same pattern as in the pear.

Potato.—Experience in the field since the publication of the paper by Hill and Roach, 1940, has proved the most useful method to be one in which the stalk of a leaflet halfway along a leaf is immersed. This results in the leaflet above and the one below becoming permeated, more heavily on the proximal than on the distal side. Slight effects appear to have resulted occasionally from the injection of water into a few leaves by this method.

Raspberry.—See Roach, 1938, p. 30 ; 1939, p. 185.

Rose.—The leaf-stalk method is the most useful and gives a similar pattern to that in the apple.

Soya bean.—See Lal. The interveinal method is the most useful.

Strawberry.—See Roach, 1938, p. 26 ; 1939, p. 179.

Tobacco.—See Lal. Both the interveinal and the leaf-stalk methods are useful.

Tomato.—See Hill and Roach, 1940, pp. 506-512.

II. INJECTION FOR CURATIVE PURPOSES.

A. Injection of liquids (cf. Roach, 1938, p. 52-60).

The method illustrated in Fig. 7 has been found satisfactory for injecting large trees. A hole is bored through the bark just down to the wood with a sharp $\frac{3}{8}$-inch Irwin bit. The hole is continued into the wood with a sharp $\frac{1}{4}$-inch similar bit. Into this hole is pushed a cylindrical plug of hardwood $\frac{3}{8}$-inch diameter, tapered at one end to a little less than $\frac{1}{4}$ inch and bored to

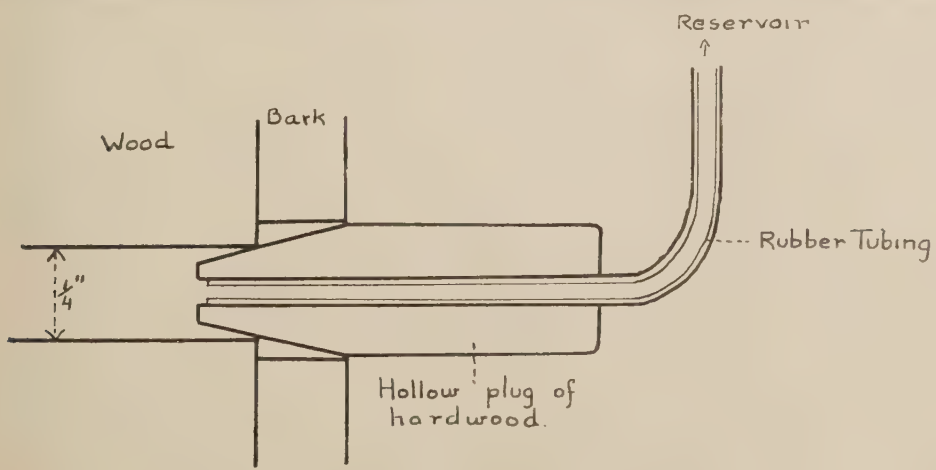

Fig. 7.

Method for leading liquid into the injection hole in the tree-trunk or branch to be injected.

take the narrow-bore rubber tubing (such as is used for bicycle tyre valves) leading to the reservoir. It is important that the boring be done by a genuine Irwin bit, kept clean and razor sharp, otherwise the cut edges will be ragged and a good joint with the plug will be impossible, nor will the hole heal rapidly and cleanly. The degree of plugging of the cells in the wood next to the bark is not found to slow up absorption appreciably except when there is only a thin ring of living wood, e.g. in a plum tree affected by Silver Leaf disease, when the method described earlier should be used (Roach, 1938, p. 53).

B. Injection of solids (cf. Roach, 1938, p. 68 ; 1939, p. 217).

The advantages of injecting a solid instead of a liquid are that little apparatus is necessary, the handling of volumes of liquid comparable with those required for spraying is avoided, and the process is completed in one operation. The use of the solid method became essential when it was found that cherry trees commonly absorbed little or no liquid (Duggan, 1943). The most serious drawback to the solid method is the amount of mechanical damage done because of the necessity of having so many holes in the trunk. Thus, about ten times as much wood is removed in making the holes for solid, as is necessary for liquid injection. Bennett's (1931) recommendation amounts to a hole of $\frac{1}{2}$-inch diameter every 3 inches of circumference of trunk or branch.

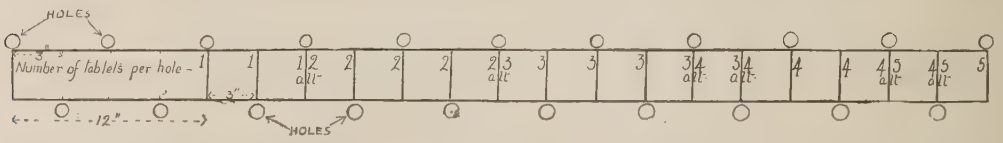

Fig. 8.

Calibrated rubber band for spacing holes for injecting a tree with substances in tablet form. The band is wrapped round the tree and the line nearest where the beginning overlaps the other end gives the number of tablets to be inserted into each hole.

This exposes the wood to risk of invasion by disease-producing organisms, and it must be reduced to a minimum. It has been found that any reduction in the number of holes results in incomplete distribution of the substance throughout the tree (Duggan, 1943).

The aim of the method now to be described is to encourage rapid and clean healing of the holes. The essentials are a clean cut and the prevention of contact between the introduced solid substance and the bark. The spacing of the holes around the trunk is most conveniently done by wrapping round it a rubber band (such as shown in Fig. 8) on which a mark has been made every 3 inches, first securing the end of it with a pin and stretching the band slightly, if necessary, to make the first mark coincide with another one. Against this latter mark will be found the number of grm. tablets of substance to be introduced into each hole. To save using tablets of smaller size to get the exact dosage for each hole when the amount comes to an odd half gm. it is permissible to use the whole number above and below alternately. It is important to note that if branches have died back the amounts injected must be reduced.

A hole of $\frac{5}{8}$-inch diameter is bored through the bark just down to the wood and is continued into the wood with a $\frac{1}{2}$-inch bit using the same centre (Fig. 9, A). As before, it has been found essential to use Irwin bits kept clean and razor sharp. A cork of $\frac{1}{2}$-inch diameter at the smaller end and $\frac{1}{4}$ inch long is placed in the end of a metal tube of $\frac{1}{2}$-inch internal and $\frac{5}{8}$-inch external diameter, and externally bevelled at the other end. The solid is obtained in 1 grm. tablets (lighter ones for smaller trees) and the requisite number are placed in the tube (Fig. 9, B). The tube is now inserted into the hole in the bark until its bevelled mouth presses on the margin of the hole in the wood (Fig. 9, C). The end of a plunger provided with a handle is now placed

against the cork and pushed home, driving tablets and cork into the hole in the wood (Fig. 9, D). The length of the plunger protruding from the handle is exactly the same as the length of the tube measured along the inside wall. The outer end of the cork, therefore, lies flush with the surface of the wood and forms a platform over which healing takes place rapidly. Holes bored

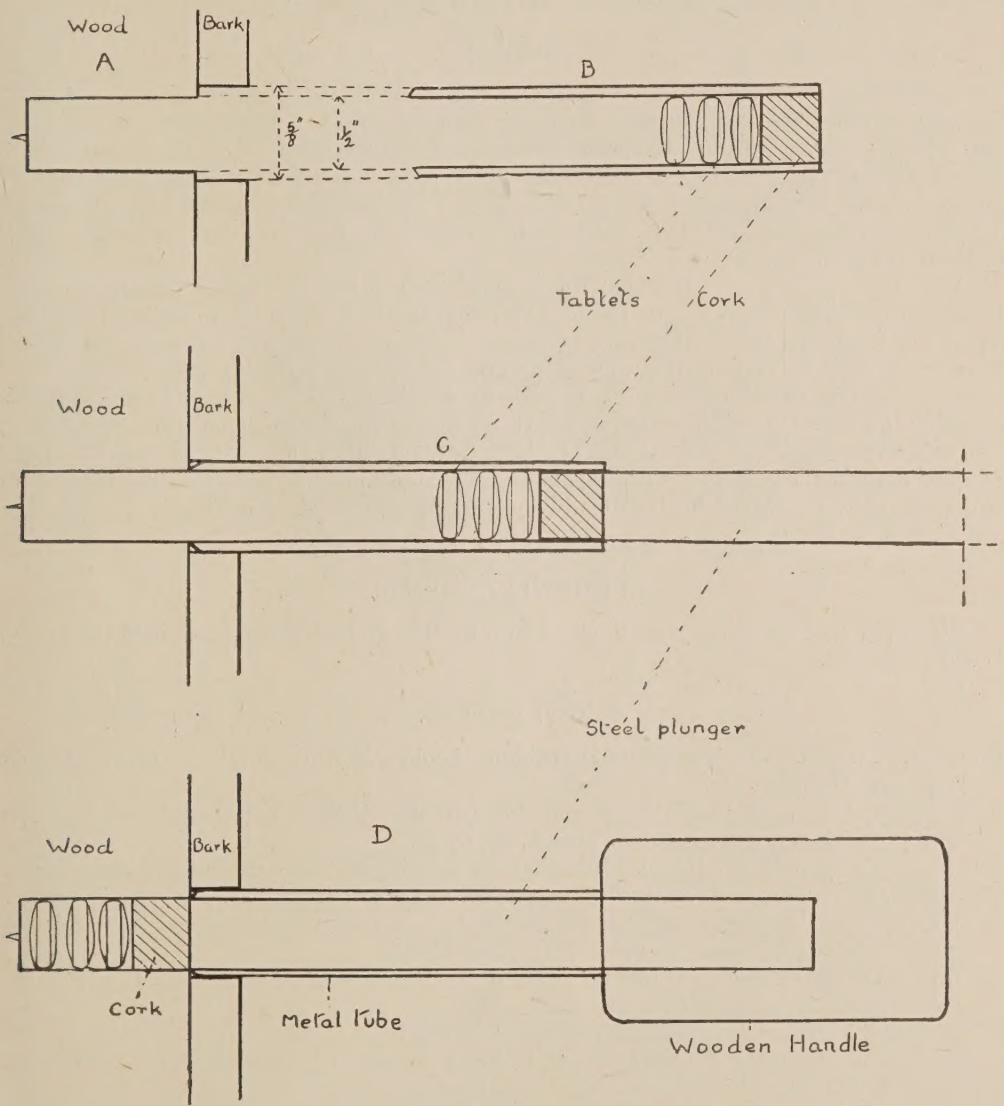

FIG. 9.*

Sectional drawings illustrating method for injecting a tree with substances in tablet form. A, Shows holes bored through bark and wood. B, Metal tube with cork and tablets in position. C, Tube inserted in hole in bark against the mouth of the hole into the wood. End of plunger ready to push cork and tablets into hole in wood. D, Cork and tablets pushed home into hole in wood.

* The implement here described can be obtained from Edward Griffin, West Malling, Kent.

in the winter or early spring heal during the succeeding summer and are barely visible by the autumn.*

Curative treatment has so far been done mainly on large trees. For small trees the size of the hole must be suitably reduced (see Bennett, 1931, p. 7, Table 3). For trees with soft bark the apparatus shown in Fig. 36 (Roach, 1938) makes the neatest holes.

SUMMARY.

Improvements in the appliances hitherto developed by one of the authors and his collaborators and used for plant injection with liquid for both diagnostic and therapeutic purposes and with solid materials for therapeutic purposes are described in detail and fully illustrated. They include the reduction in weight of the small containers employed for interveinal and leaf-stalk injection by the substitution of cellophane for glass. Special tools for making the cellophane cups are described and illustrated and the sources from which the requisite materials can be obtained are mentioned.

Brief notes are given on the experience gained both in this country and South Africa in the use of injection methods with twenty-five different kinds of plants, including fruit trees and bushes, some forage plants, hops, potatoes, tomatoes, tobacco and a few others. Paged references are given to information contained in previous papers on the subject.

In connection with injection for curative purposes, an improved method for the injection of solids into a tree is described and figured. It consists in drilling a small hole through the bark, continuing it at a slightly decreased diameter into the wood, introducing with the help of a metal tube and plunger the necessary chemicals in tablet form into the wood and sealing them there with a cork disc, over which the bark readily heals. By this method a minimum of mechanical damage is done to the tree.

ACKNOWLEDGMENTS.

Figs. 2, 3, 4, 5, 11 and 12 were kindly drawn by Miss M. E. Bunyard, to whom the writers are duly grateful.

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* Mr. C. R. Thompson, Assistant Advisory Officer in Commercial Fruit Growing to the Kent Education Committee, has used an apparatus differing slightly from the one just described. A description of this has appeared in the *Market Grower* for Oct. 14, 1943, pp. 12-13 and for Oct. 21, p. 15, since this paper went to press.

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